08-01-00

BOX - PATENT

Attorney's Docket No.: U 012858-1

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Box Patent Application
Assistant Commissioner for Patents
Washington, D.C. 20231

NEW APPLICATION TRANSMITTAL

Transmitted herewith for filing is the patent application of Inventors:

1. ANAND C. **BURMAN**
2. SUDHANAND **PRASAD**
3. RAMA **MUKHERJEE**
4. MANU **JAGGI**
5. ANU T. **SINGH**
6. RAJAN **SHARMA**

WARNING: *The Declaration must name all of the actual inventor(s).*

For (title):
NOVEL PEPTIDE FOR TREATMENT OF CANCER

1. Type of Application

This new application is for a(n) (check one applicable item below):

- ☑ Original (nonprovisional)
- ☐ Design
- ☐ Plant

WARNING: ***Do not*** *use this transmittal for a completion in the U.S. of an International Application under 35 U.S.C. 371(c)(4) unless the International Application is being filed as a divisional, continuation or continuation-in-part application.*

CERTIFICATION UNDER 37 CFR 1.10

I hereby certify that this New Application Transmittal and the documents referred to as enclosed therein are being deposited with the United States Postal Service on this date **JULY 31, 2000 in an envelope as "Express Mail Post Office to Addressee" Mailing Label Number EL386270297US addressed to the: Assistant Commissioner of Patents, Washington, D.C. 20231**

GERALDINE MARTI
(type or print name of person mailing paper)

Geraldine Marti
(Signature of person mailing paper)

NOTE: ***Each paper or fee referred to as enclosed herein has the number of the "Express Mail" mailing label placed thereon prior to mailing. 37 CFR 1.10(b).***

WARNING: ***Certificate of mailing (first class) or facsimile transmission procedures of 37 CFR 1.8 cannot be used to obtain a date of mailing or transmission for this correspondence.***

WARNING: ***Do not*** *use this transmittal for the filing of a provisional application.*

2. Benefit of Prior U.S. Application(s) (35 U.S.C. 119(e), 120, or 121)

NOTE: If the new application being transmitted is a divisional, continuation or a continuation-in-part of a parent case, or where the parent case is an International Application which designated the U.S., or benefit of a prior provisional application is claimed, then check the following item and complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

WARNING: *If an application claims the benefit of the filing date of an earlier filed application under 35 U.S.C. 120, 121 or 365(c), the 20-year term of that application will be based upon the filing date of the earliest U.S. application that the application makes reference to under 35 U.S.C. 120, 121 or 365(c). (35 U.S.C. 154(a)(2) does not take into account, for the determination of the patent term, any application on which priority is claimed under 35 U.S.C. 119, 365(a) or 365(b).) For a c-i-p application, applicant should review whether any claim in the patent that will issue is supported by an earlier application and, if not, the applicant should consider canceling the reference to the earlier filed application. The term of a patent is not based on a claim-by-claim approach. See Notice of April 14, 1995, 60 Fed. Reg. 20,195, at 20,205.*

WARNING: *When the last day of pendency of a provisional application falls on a Saturday, Sunday, or Federal holiday within the District of Columbia, any nonprovisional application claiming benefit of the provisional* ***must*** *be filed prior to the Saturday, Sunday or Federal holiday within the District of Columbia. See 37 C.F.R. § 1.78(a)(3).*

☐ The new application being transmitted claims the benefit of prior U.S. application(s) and enclosed are ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

NOTE: If one of the following 3 items apply, then complete and attach ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF A PRIOR U.S. APPLICATION CLAIMED and a NOTIFICATION IN PARENT APPLICATION OF THE FILING OF THIS CONTINUATION APPLICATION.

☐ Divisional.

☐ Continuation.

☐ Continuation-in-Part (C-I-P).

3. Papers Enclosed That Are Required For Filing Date Under 37 CFR 1.53 (Regular) or 37 CFR 1.153 (Design) Application

12 Pages of specification

2 Pages of claims

1 Pages of Abstract

1 Sheets of drawing

☐ formal

☐ informal

WARNING: ***DO NOT*** *submit original drawings. A high quality copy of the drawings should be supplied when filing a patent application. The drawings that are submitted to the Office must be on strong, white, smooth, and non-shiny paper and meet the standards according to § 1.84. If corrections to the drawings are necessary, they should be made to the original drawing and a high-quality copy of the corrected original drawing then submitted to the Office. Only one copy is required or desired. Comments on proposed new 37 CFR 1.84. Notice of March 9, 1988 (1990 O.G. 57-62).*

NOTE: "Identifying indicia, if provided, should include the application number or the title of the invention, inventor's name, docket number (if any), and the name and telephone number of a person to call if the Office is unable to match the drawings to the proper application. This information should be placed on the back of each sheet of drawing a minimum distance of 1.5 cm. (⅝ inch) down from the top of the page." 37 C.F.R. 1.84(c).

(complete the following, if applicable)

☐ The enclosed drawing(s) are photograph(s), and there is also attached a "PETITION TO ACCEPT PHOTOGRAPH(S) AS DRAWING(S)". 37 C.F.R. 1.84(b).

4. Additional papers enclosed

☑ Preliminary Amendment

☑ Information Disclosure Statement (37 CFR 1.98)

☑ Form PTO-1449

☑ Citations

☐ Declaration of Biological Deposit

☑ Submission of "Sequence Listing," computer readable copy and/or amendment pertaining thereto for biotechnology invention containing nucleotide and/or amino acid sequence.

☐ Authorization of Attorney(s) to Accept and Follow Instructions from Representative

☐ Special Comments

☐ Other

5. Declaration or oath

☑ Enclosed

executed by *(check **all** applicable boxes)*

☑ inventors.

☐ legal representative of inventors. 37 CFR 1.42 or 1.43

☐ joint inventor or person showing a proprietary interest on behalf of inventor who refused to sign or cannot be reached.

☐ This is the petition required by 37 CFR 1.47 and the statement required by 37 CFR 1.47 is also attached. *See item 13 below for fee.*

☐ Not Enclosed.

WARNING: *Where the filing is a completion in the U.S. of an International Application but where a declaration is not available or where the completion of the U.S. application contains subject matter in addition to the International Application the application may be treated as a continuation or continuation-in-part, as the case may be, utilizing ADDED PAGE FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION CLAIMED.*

☐ Application is made by a person authorized under 37 CFR 1.41(c) on behalf of *all the above named inventors.* (The declaration or oath, along with the surcharge required by 37 CFR 1.16(e) can be filed subsequently).

NOTE: It is important that all the correct inventor(s) are named for filing under 37 CFR 1.41(c) and 1.53(b).

☐ Showing that the filing is authorized. *(Not required unless called into question. 37 CFR 1.41(d).)*

6. Inventorship Statement

WARNING: *If the named inventors are each not the inventors of all the claims an explanation, including the ownership of the various claims at the time the last claimed invention was made, should be submitted.*

The inventorship for all the claims in this application are:

☐ The same

☐ Not the same. An explanation, including the ownership of the various claims at the time the last claimed invention was made,

7. Language

NOTE: An application including a signed oath or declaration may be filed in a language other than English. A verified English translation of the non-English language application and the processing fee of $130.00 required by 37 CFR 1.17(k) is required to be filed with the application or within such time as may be set by the Office. 37 CFR 1.52(d).

NOTE: A non-English oath or declaration in the form provided or approved by the PTO need not be translated. 37 CFR 1.69(b).

☑ English

☐ non-English

☐ the attached translation is a verified translation. 37 CFR 1.52(d).

8. Assignment

☑ An assignment of the invention to DABUR RESEARCH FOUNDATION

☑ is attached. A separate ☑ "COVER SHEET FOR ASSIGNMENT (DOCUMENT) ACCOMPANYING NEW PATENT APPLICATION" or ☐ FORM PTO 1595 is also attached.

☐ will follow.

NOTE: "If an assignment is submitted with a new application, send two separate letters—one for the application and one for the assignment." Notice of May 4, 1990 (1114 O.G. 77-78).

***WARNING:** A newly executed "CERTIFICATE UNDER 37 CFR 3.73(b)" must be filed when a continuation-in-part application is filed by an assignee. Notice of April 30, 1993. 1150 O.G. 62-64.*

9. Certified Copy

Certified copy of application

Country	Appln. No.	Filed

from which priority is claimed

☐ is attached.

☐ will follow.

NOTE: The foreign application forming the basis for the claim for priority must be referred to in the oath or declaration. 37 CFR 1.55(a) and 1.63.

NOTE: This item is for any foreign priority for which the application being filed directly relates. If any parent U.S. application or International Application from which this application claims benefit under 35 U.S.C. 120 is itself entitled to priority from a prior foreign application then complete item 18 on the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED.

10. Fee Calculation (37 CFR 1.16)

A. ☑ Regular Application

Claims as Filed

Number Filed	Number Extra	Rate	Basic Fee 37 CFR 1.16(a) $690.00
Total Claims (37 CFR 1.16(c))	13 - 20 = 0	x $ 18.00	
Independent Claims (37 CFR 1.16(b))	3 - 3 = 0	x $ 78.00	
Multiple dependent claim(s), if any (37 CFR 1.16(d))		+ $ 260.00	

☐ Amendment cancelling extra claims enclosed.

☐ Amendment deleting multiple-dependencies enclosed.

☐ Fee for extra claims is not being paid at this time.

NOTE: If the fees for extra claims are not paid on filing they must be paid or the claims cancelled by amendment, prior to the expiration of the time period set for response by the Patent and Trademark Office in any notice of fee deficiency. 37 CFR 1.16(d).

Filing Fee Calculation $ 690.00

B. ☐ Design application ($310.00 — 37 CFR 1.16(f))

Filing Fee Calculation $

C. ☐ Plant application ($480.00 — 37 CFR 1.16(g))

Filing Fee Calculation $

11. Small Entity Statement(s)

☐ Verified Statement(s) that this is a filing by a small entity under 37 CFR 1.9 and 1.27 is(are) attached or has been filed.

Filing Fee Calculation (50% of **A**, **B** or **C** above) $

NOTE: Any excess of the full fee paid will be refunded if a verified statement and a refund request are filed within 2 months of the date of timely payment of a full fee. 37 CFR 1.28(a).

12. Request for International-Type Search (37 CFR 1.104(d)) *(Complete, if applicable)*

☐ Please prepare an international-type search report for this application at the time when national examination on the merits takes place.

13. Fee Payment Being Made At This Time

☐ Not Enclosed

☐ No filing fee is to be paid at this time. *(This and the surcharge required by 37 CFR 1.16(e) can be paid subsequently.)*

☑ Enclosed

☑ basic filing fee $ 690.00

☑ Recording assignment
($40.00; 37 CFR 1.21(h)) (See attached "COVER SHEET FOR ASSIGNMENT ACCOMPANYING NEW APPLICATION.")

☐ Petition fee for filing by other than all the inventors or person on behalf of the inventor where inventor refused to sign or cannot be reached.
($130.00; 37 CFR 1.47 and 1.17(h)) $

☐ For processing an application with a specification in a non-English language.
($130.00; 37 CFR 1.52(d) and 1.17(k)) $

☐ Processing and retention fee
($130.00; 37 CFR 1.53(d) and 1.21(l))

☐ Fee for international-type search report
($40.00; 37 CFR 1.21(e)). $

NOTE: 37 CFR 1.21(l) establishes a fee for processing and retaining any application which is abandoned for failing to complete the application pursuant to 37 CFR 1.53(d) and this, as well as the changes to 37 CFR 1.53 and 1.78, indicate that in order to obtain the benefit of a prior U.S. application, either the basic filing fee must be paid or the processing and retention fee of §1.21(l) must be paid within 1 year from notification under §53(d).

Total fees enclosed $ 690.00

14. Method of Payment of Fees

☑ Check in the amount of $ 690.00

☐ Charge Account No. 12-0425 in the amount of $

A duplicate of this transmittal is attached.

NOTE: Fees should be itemized in such a manner that it is clear for which purpose the fees are paid. 37 CFR 1.22(b).

15. Authorization to Charge Additional Fees

WARNING: *If no fees are to be paid on filing, the following items should not be completed.*

WARNING: *Accurately count claims, especially multiple dependent claims, to avoid unexpected high charges, if extra claim charges are authorized.*

☑ The Commissioner is hereby authorized to charge the following additional fees by this paper and during the entire pendency of this application to Account No. 12-0425.

☑ 37 CFR 1.16(a), (f) or (g) (filing fees)

☐ 37 CFR 1.16(b), (c) and (d) (presentation of extra claims)

NOTE: Because additional fees for excess or multiple dependent claims not paid on filing or on later presentation must only be paid or these claims cancelled by amendment prior to the expiration of the time period set for response by the PTO in any notice of fee deficiency (37 CFR 1.16(d)), it might be best not to authorize the PTO to charge additional claim fees, except possibly when dealing with amendments after final action.

☐ 37 CFR 1.16(e) (surcharge for filing the basic filing fee and/or declaration on a date later than the filing date of the application)

☑ 37 CFR 1.17 (application processing fees)

WARNING: *While 37 CFR 1.17(a), (b), (c) and (d) deal with extensions of time under §1.136(a), this authorization should be made only with the knowledge that: "Submission of the appropriate extension fee under 37 C.F.R. 1.136(a) is to no avail unless a request or petition for extension is filed." (Emphasis added). Notice of November 5,1985 (1060 O.G. 27)*

☑ 37 CFR 1.18 (issue fee at or before mailing of Notice of Allowance, pursuant to 37 CFR 1.311(b))

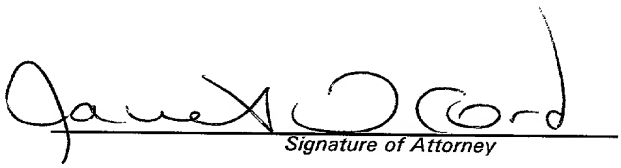
NOTE: Where an authorization to charge the issue fee to a deposit account has been filed before the mailing of a Notice of Allowance, the issue fee will be automatically charged to the deposit account at the time of mailing the notice of allowance. 37 CFR 1.311(b).

NOTE: 37 CFR 1.28(b) requires "Notification of any change in loss of entitlement to small entity status must be filed in the application ... prior to paying, or at the time of paying, ... issue fee". From the wording of 37 CFR 1.28(b): (a) notification of change of status must be made even if the fee is paid as "other than a small entity" and (b) no notification is required if the change is to another small entity.

16. Instructions As To Overpayment

☑ credit Account No. 12-0425

☐ refund



Signature of Attorney

Reg. No. 33,778

Tel. No. (212) 708-1935

Janet I. Cord
Ladas & Parry
26 West 61 Street
New York, NY 10023

☑ **Incorporation by reference of added pages**

(Check the following item if the application in this transmittal claims the benefit of prior U.S. application(s) (including an international application entering the U.S. stage as a continuation, divisional or C-I-P application) and complete and attach the ADDED PAGES FOR NEW APPLICATION TRANSMITTAL WHERE BENEFIT OF PRIOR U.S. APPLICATION(S) CLAIMED)

☐ Plus Added Pages for New Application Transmittal Where Benefit of Prior U.S. Application(s) Claimed

Number of pages added ___

☐ Plus Added Pages for Papers Referred to in Item 4 Above

Number of pages added ___

☑ Plus "Assignment Cover Letter Accompanying New Application"

Number of pages added 4

☐ **Statement Where No Further Pages Added**

(If no further pages form a part of this Transmittal, then end this Transmittal with this page and check the following item:)

☐ This transmittal ends with this page.

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: ANAND C. **BURMAN**, et al

For: NOVEL PEPTIDE FOR TREATMENT OF CANCER

Attorney Docket No.: U 012858-1

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

PRELIMINARY AMENDMENT

Please insert the attached sequence listing after page 12 of the specification.

Respectfully submitted,

JANET I. CORD
LADAS & PARRY
26 WEST 61ST STREET
NEW YORK, NEW YORK 10023
REG.NO.33778(212)708-1935

CERTIFICATE UNDER 37 CFR 1.10

I hereby certify that this paper is being deposited with the United States Postal Service on this date JULY 31, 2000 in an envelope as "EXPRESS MAIL POST OFFICE TO ADDRESSEE" Mailing Label Number EL386270297US addressed to the: Assistant Commissioner for Patents, Washington, D.C. 20231

GERALDINE MARTI
(Type or print name of person mailing paper)

(Signature of person mailing paper)

NOTE: Each paper or fee referred to as enclosed herein has the number of the "EXPRESS MAIL" mailing label place thereon prior to mailing 37 CFR 1.16(b).

NOVEL PEPTIDES FOR TREATMENT OF CANCER

FIELD OF INVENTION

This invention relates to novel antiproliferative and anti secretory peptides that are inhibitory to vasoactive intestinal peptide receptor and are useful in the treatment of cancer. The invention particularly relates to the synthesis of lipid-peptide conjugates containing fatty acids of different sizes, which inhibits the binding of VIP to its receptors. The invention encompasses methods for generation of these peptides, composition containing these peptides and the pharmacological applications of these peptides especially in the treatment and prevention of cancer.

BACKGROUND OF THE INVENTION

Vasoactive intestinal peptide (VIP) is a 28-amino acid neuropeptide, which was first isolated from the porcine intestine (Said, S. I. and Mutt, V. , Science, 169, 1217-1218, 1970.) VIP acts as growth factor and plays dominant autocrine and paracrine role in the sustained proliferation of cancer cells. (Said, S.I., Peptides, 5, 143-150, 1984.) Gozes et al. have shown that VIP can serve as autocrine growth factor in lung tumors. (Gozes et al. Biomed. Res. 13 (suppl.2) 37, 1992).

The peptide sequence Leu-Met-Tyr-Pro-Thr-Tyr-Leu-Lys (SEQ ID NO: 1) is reported to be receptor binding inhibitor of vasoactive intestinal peptide (Said, & Mutt, Ann. N.Y. Acad. Sci., 1, 527, 1988). The role this octapeptide as VIP receptor binding inhibitor has been described in the U.S Patent 5,217,953. In our U.S. Patent Application 08/727,679 we have described the anti cancer role of this VIP binding receptor inhibitor in combination with other neuropeptide analogs. In another U.S. Patent Application 09/248382 we have described the novel analogs of this VIP receptor binding inhibitor incorporating dialkylated amino acids. Keeping in view that lipophilization of bioactive peptides improves their stability, bioavailability and the ability to permeate biomembranes (Dasgupta, P. et al.; 1999, Pharmaceutical Res. 16, 1047-1053; Gozes, I. et al., 1996, Proc. Natl. Acad. Sci. USA, 93, 427-432.), in the present invention we have synthesized lipid conjugates of the peptide sequence Leu-Met-Tyr-Pro-Thr-Tyr-Leu-Lys (SEQ ID NO: 1) using fatty acids of different sizes, C2- C16 carbon atoms, at the N-terminal site of the peptide. Throughout the application the following abbreviation are used with

the following meanings:

BOP: Benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium hexofluorophospate

PyBOP: Benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexofluorophospate

HBTU: O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexofluoro-phosphate

TBTU: 2-(1H-Benzotriazole-lyl)-1,1,3,3,-tetramethyluronium tetrafluoroborate

HOBt: 1-Hydroxy Benzotriazole

DCC: Dicyclohexyl carbodiimide

DIPCD: Diisopropyl carbodiimide

DIEA: Diisopropyl ethylamine

DMAP: 4-Dimethylamino pyridine

DMF: Dimethyl formamide

DCM: Dichloromethane

NMP: N-Methyl-2-pyrrolidinone

TFA: Trifluoroacetic acid

Throughout the specification and claims, the amino acids residues are designated by their standard abbreviations. Amino acids denote L-configuration unless otherwise indicated by D or DL appearing before the symbol and separated from it by hyphen.

Throughout the specification and claims, the amino acids residues are designated by their standard abbreviations. Amino acids denote L-configuration unless otherwise indicated by D or DL appearing before the symbol and separated from it by hyphen.

SUMMARY OF THE INVENTION

The present invention relates to peptides of the following general formula

X-Leu-Met-Tyr-Pro-Thr-Tyr-Leu-Lys-Y

wherein,

X is acetyl or straight, branched, or cyclic alkanoyl group of from 3 -

16 carbon atoms.

Y is a carboxy terminal residue selected from OH or amino; or a pharmaceutical acceptable salt of the peptides.

BRIEF DESCRIPTION OF THE FIGURE

Figure 1 shows the anti-cancer activity of the peptide DT-B1 on PCT xenograft.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to peptides of the following general formula

X-Leu-Met-Tyr-Pro-Thr-Tyr-Leu-Lys-Y

wherein,

X is acetyl or straight, branched, or cyclic alkanoyl group of from 3 - 16 carbon atoms.

Y is a carboxy terminal residue selected from OH or amino; or a pharmaceutical acceptable salt of the peptides.

The preferred alkanoyl groups are acetyl, n-butanoyl, n-hexanoyl, n-octanoyl, lauroyl, myristoyl, palmitoyl, isohexanoyl, cyclohexanoyl, cyclopentylcarbonyl, n-heptanoyl, n-decanoyl, n-undecanoyl, and 3,7-dimethyloctanoyl.

Salts encompassed within the term "pharmaceutically acceptable salts" refer to non-toxic salts of the compounds of this invention. Representative salts and esters include following: acetate, ascorbate, benzenesulfonate, benzoate, bicarbonate, bisulfate, bitartrate, borate, camsylate, carbonate, citrate, dihydrochloride, methanesulfonate, ethanesulfonate, ρ-toluenesulfonate, cyclohexylsulfamate, quinate, edetate, edisylate, estolate, esylate, fumarate, gluconate, glutamate, glycerophophates, hydrobromide, hydrochloride, hydroxy-naphthoate, lactate, lactobionate, laurate, malate, maleate, mandelate, mesylate, mucate, napsylate, nitrate, n-methylglucamine, oleate, oxalate, palmoates, pamoate (embonate), palmitate, pantothenate, perchlorates, phosphate/diphosphate, polygalacturonate, salicylates, stearate, succinates, sulfate, sulfamate, subacetate, succinate, tannate, tartrate, tosylate, trifluoroacetate and valerate.

Other salts include Ca, Li, Mg, Na, and K salts; salts of amino acids

such as lysine or arginine; guanidine, diethanolamine or choline; ammonium, substituted ammonium salts or aluminum salts.

The salts are prepared by conventional methods.

The preferred lipo-peptide analogs are:

Acetyl-Leu-Met-Tyr-Pro-Thr-Tyr-Leu-Lys-OH (DT-Al) (SEQ ID NO: 2)

n-Butanoyl-Leu-Met-Tyr-Pro-Thr-Tyr-Leu-Lys-OH (DT-B1) (SEQ ID NO: 3)

n-Octanoyl-Leu-Met-Tyr-Pro-Thr-Tyr-Leu-Lys-OH (DT-01) (SEQ ID NO: 4)

Myristoyl-Leu-Met-Tyr-Pro-Thr-Tyr-Leu-Lys-OH(DT-M1) (SEQ ID NO: 5)

Palmitoyl- Leu-Met-Tyr-Pro-Thr-Tyr-Leu-Lys-OH (DT-P1) (SEQ ID NO: 6)

The novel compounds of the present invention have important pharmacological applications. They are potent anti-neoplastic agents and thereby possess therapeutic potential in a number of human cancers.

The lipopeptides in the present invention have been generated by using solid phase techniques or by a combination of solution phase procedures and solid phase techniques or by fragment condensation. The methods for the chemical synthesis of polypeptides are well known in the art (Stewart and Young, 1969 Solid Phase Synthesis, W.H. Freeman Co.).

In a preferred embodiment of the present invention the peptides were synthesized using the Fmoc strategy, on a semi automatic peptide synthesizer (CS Bio, Model 536), using optimum side chain protection. The peptides were assembled from C-terminus to N-terminus. Peptides amidated at the carboxy-terminus were synthesized using the Rink Amide resin. The loading of the first Fmoc protected amino acid was achieved via an amide bond formation with the solid support, mediated by Diisopropylcarbodiimide (DIPCDI) and HOBt. Substitution levels for automated synthesis were preferably between 0.2 and 0.6 mmole amino acid per gram resin. The steps involved in the synthesis of the peptide analogs employed the following protocol:

TABLE I

STEP	REAGENT	MIX TIME (MIN)	NO. OF CYCLES
1.	Methylene chloride	1	2
2.	Dimethyl formamide	1	1
3.	20 % Piperidine in Dimethyl formamide	1	1
4.	20 % Piperidine in Dimethyl formamide	29	1
5.	Dimethyl formamide	1	3
6.	Isopropanol	1	2
7.	Methylene chloride	1	2
8.	Amino Acid	Variable	1
9.	Dimethyl formamide	1	2
10.	Stop or Return for next cycle		

In a particularly preferred embodiment of the present invention the following chemical moieties were used to protect reactive side chains of the peptides during the synthesis procedure:

The N-terminal amino group was protected by 9-fluorenylmethoxy-carbonyl (Fmoc) group. The hydroxyl groups of Threonine and Tyrosine were preferably protected by t-butyl group (tBu). Leu, Met and Pro were used unprotected.

In a preferred embodiment of the invention, 2-8 equivalents of Fmoc protected amino acid per resin nitrogen equivalent were used. The activating reagents used for coupling amino acids to the resin, in solid phase peptide synthesis, are well known in the art. These include DCC, DIPCDI, DIEA, BOP, PyBOP, HBTU, TBTU, and HOBt. Preferably, DCC or DIPCDI / HOBt or HBTU/HOBt and DIEA were used as activating reagents in the coupling reactions. The protected amino acids were either activated in situ or added in the form of preactivated esters known in the art such as N-hydroxy succinamide esters, pentafluorophenyl esters etc. The coupling reaction was carried out in DMF, DCM or NMP or a mixture of these solvents and was monitored by Kaiser test [Kaiser et al., Anal. Biochem., 34,

595-598 (1970)]. In case of a positive Kaiser test, the appropriate amino acid was re-coupled using freshly prepared activated reagents.

After the assembly of the peptide analog was completed, the amino-terminal Fmoc group was removed using steps 1-6 of the above protocol and then the peptide-resin was washed with methanol and dried. The analogs were then deprotected and cleaved from the resin support by treatment with trifluoroacetic acid, crystalline phenol, ethanedithiol, thioanisole and de-ionized water for 1.5 to 5 hours at room temperature. The crude peptide was obtained by precipitation with cold dry ether, filtered, dissolved, and lyophilized.

The resulting crude peptide was purified by preperative high performance liquid chromatography (HPLC) using a LiChrOCART® C_{18} (250. Times. 10) reverse phase column (Merck, Darmstadt, Germany) on a Preparative HPLC system (Shimadzu Corporation, Japan) using a gradient of 0.1 % TFA in acetonitrile and water. The eluted fractions were reanalyzed on Analytical HPLC system (Shimadzu Corporation, Japan) using a C_{18} LiChrospher®, WP-300 (300 X 4) reverse-phase column. Acetonitrile was evaporated and the fractions were lyophilized to obtain the pure peptide. The identity of each peptide was confirmed by electron-spray mass spectroscopy.

An analog of the present invention can be made by exclusively solid phase techniques, by partial solid phase/solution phase techniques and/or fragment condensation. Preferred, semi-automated, stepwise solid phase methods for synthesis of peptides of the invention are provided in the examples discussed in the subsequent section of this document.

The present invention will be further described in detail with reference to the following examples, as will be appreciated by a person skilled in the art is merely illustrative and should not be construed as limiting. Various other modifications of the invention will be possible without departing from the spirit and scope of the present invention.

Synthesis of peptides

First loading on Wang Resin

A typical preparation of the Fmoc-Lys-Wang Resin was carried out using 1.0 g of 4-Hydroxymethylphenoxy Resin 1% DVB cross-linked resin (0.7

mM/g) (100-200 mesh), procured from Advanced Chemtech, Louisville, KY, U.S.A. Swelling of the resin was typically carried out in dichloromethane measuring to volumes 10-40ml/g resin. The resin was allowed to swell in methylene chloride (2 X 25 ml, for 10 min.). It was washed once in dimethylformamide (DMF) for 1 min. All solvents in the protocol were added in 20 ml portions per cycle. For loading of the first amino acid on hydroxyl group of the resin, the first amino acid, was weighed in three to six fold excess, along with a similar fold excess of HOBt, in the amino acid vessel of the peptide synthesizer. These were dissolved in dimethylformamide (A.C.S. grade) (J.T.Baker,, New Jersey, U.S.A.) and activated with DIPCDI and 4-dimethyl amino pyridine (DMAP),just prior to the addition to the resin in the reaction vessel of the peptide synthesizer. The coupling reaction was carried out for a period ranging from 6 hours. The loading of the amino acid on the resin was confirmed by the weight gain of the resin. The loading efficiency was ascertained by the increase of weight of the resin after the addition of the amino acid.

EXAMPLE 1

Synthesis of Acetyl-Leu-Met-Tyr-Pro-Thr-Tyr-Leu-Lys-OH (DT-A1) (SEQ ID NO: 2)

The synthesis of peptide DT-A1 was initiated by using resin loaded with Fmoc-Lys-OH as prepared above on 1g scale. It was subjected to stepwise deprotection and coupling steps as in steps 1-10 of the synthesis cycle. In each coupling reaction, a four-fold excess of appropriate Fmoc amino acid, DIPCDI and HOBt were used. The average coupling time for each amino acids was between 2-5 hrs. On completion of synthesis and removal of the N-terminal Fmoc protecting group (steps 1-6 of the synthesis cycle), the peptideresin was washed twice with methanol. It was further coupled with acetic anhydride in DMF using DIPCDI and HOBt as coupling agents. This was subjected to cleavage in a cleavage mixture consisting of trifluoroacetic acid and scavengers, crystalline phenol, thioanisole, ethanedithol and water for a period of 1-4 hours at room temperature with continuous stirring. The peptide was precipitated using cold dry ether to obtain the crude peptide. The crude peptide was purified on a C_{18} preparative reverse phase HPLC column (250 X 10) on a gradient system comprising acetonitrile and water in

0.1% TFA as described previously, in the art. The prominent peaks were collected and lyophilized, reanalyzed on analytical HPLC and subjected to mass spectrometry. There was a good agreement between the observed molecular weight and calculated molecular weight (calculated mass = ~ 1070; observed mass = 1071.1). The pure peptide was then used for bioassays.

EXAMPLE 2

Synthesis of n-Butanoyl-Leu-Met-Tyr-Pro-Thr-Tyr-Leu-Lys-OH (DT-B1)

SEQ ID NO: 3

The above peptide sequence was synthesized on resin in a similar way as described in Example 1 except n-butyric anhydride is used in place of acetic anhydride. The final purified peptide was further analyzed by mass spectroscopy. The calculated mass and observed mass was in good agreement (calculated mass = ~ 1098, observed mass = 1099.3).

EXAMPLE 3

Synthesis of n-Octanoyl-Leu-Met-Tyr-Pro-Thr-Tyr-Leu-Lys-OH (DT-O1)

(SEQ ID NO: 4)

The above peptide sequence was synthesized on resin in a similar way as described in Example 1 except octanoic acid is used in place of acetic anhydride. The final purified peptide was further analyzed by mass spectroscopy. The calculated mass is ~ 1154 and observed mass is 1155.2.

EXAMPLE 4

Synthesis of Myristoyl -Leu-Met-Tyr-Pro-Thr-Tyr-Leu-Lys-OH (DT-M1)

(SEQ ID NO: 5)

The above peptide sequence was synthesized on resin in a similar way as described in Example 1 except myristic acid is used in place of acetic anhydride. The final purified peptide was further analyzed by mass spectroscopy (calculated mass = ~ 1238, observed mass = 1239.6).

EXAMPLE 5

Synthesis of Palmitoyl- Leu-Met-Tyr-Pro-Thr-Tyr-Leu-Lys-OH (DT-P1)

(SEQ ID NO: 6)

The above peptide sequence was synthesized on resin in a similar way as described in Example 1 except palmitic acid is used in place of acetic

anhydride. The final purified peptide was further analyzed by mass spectroscopy (calculated mass = ~ 1262, observed mass = 1263.4).

EXAMPLE 6

The cytotoxic effect of Lipo peptide analogs, DT-A1 (SEQ ID NO: 2), DT-B1 (SEQ ID NO: 3), DT-O1 (SEQ ID NO: 4), DT-M1 (SEQ ID NO: 5) and DT-P1 (SEQ ID NO: 6) was studied by MTT assay which is based on the principle of uptake of MTT [3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide], a tetrazolium salt by the metabolically active cells where it is metabolized by active mitochondria into a blue colored formazan product which can be read spectrophotometrically. Briefly, tumor cells PTC (primary colon) KB (Oral squamous), U87MG (Glioblastoma), HBL100 (Breast), HeP2 (Laryngeal), ECV304 (Endothelial), PA-1 (Ovary) and L132 (Lung) were incubated with the peptide analogs for 48 hours at 37°C in a 96-well culture plate, followed by the addition of 100 μg MTT and further incubation of 1 hour. The formazan crystals formed inside the cells were dissolved with a detergent comprising 10% Sodium dodecyl sulfate and 0.01 N HCl and optical density read on a multiscan ELISA reader. The optical density was directly proportional to the number of proliferating and metabolically active cells. Percent cytotoxicity of peptide analogs is shown in the following Tables.

DT-A1

	Percentage cytotoxicity at different concentrations			
Cell Line	1μM	100n M	10 nM	1nM
KB	21.0 ± 2.3	26.9 ± 2.1	31.0 ± 2.1	25.9 ± 1.6
U87MG	21.9 ± 1.4	26.9 ± 1.5	29.9 ± 2.2	10.4 ± 1.3
HBL100	28.9 ± 1.2	30.5 ± 1.3	31.9 ± 3.5	19.9 ± 3.5
HeP2	19.7 ± 1.1	21.9 ± 2.7	39.9 ± 1.8	14.9 ± 2.2
L132	12.9 ± 2.4	14.6 ± 3.1	26.4 ± 3.2	13.9 ± 2.9
PA-1	6.9 ± 3.2	18.5 ± 2.3	24.9 ± 2.6	21.9 ± 1.5
ECV304	10.8 ± 3.4	22.0 ± 2.3	16.5 ± 3.4	8.0 ± 2.5

DT-B1

	Percentage cytotoxicity at different concentrations			
Cell Line	1μM	100n M	10 nM	1nM
PTC	31 ± 1.5	48 ± 1.7	44 ± 1.3	36 ± 1.0
KB	14.8 ± 2.3	18.9 ± 3.2	25.9 ± 4.1	21.9 ± 0.5
U87MG	20.6 ± 1.7	30.7 ± 1.7	39.7 ± 2.7	33.9 ± 0.6
HBL100	32.8 ± 2.2	33.9 ± 2.8	34.8 ± 1.8	33.0 ± 1.5
HeP2	12.9 ± 4.4	14.9 ± 5.3	22.9 ± 1.6	8.7 ± 1.5
L132	11.8 ± 2.3	10.7 ± 2.6	26.8 ± 1.9	13.9 ± 1.9
PA-1	33.7 ± 1.6	38.5 ± 1.6	45.8 ± 1.2	43.9 ± 2.9
ECV304	25.8 ± 2.3	31.9 ± 2.8	19.9 ± 4.2	18.7 ± 5.3

DT-O1

	Percentage cytotoxicity at different concentrations			
Cell Line	1μM	100n M	10 nM	1nM
PTC	29 ± 5.5	33 ± 3.2	32 ± 0.3	28 ± 5.4
KB	23.9 ± 1.3	26.9 ± 2.2	34.9 ± 3.1	21.0 ± 0.6
U87MG	21.9 ± 1.5	28.6 ± 1.2	38.7 ± 1.7	37.0 ± 1.6
HBL100	27.4 c± 2.7	32.8 ± 2.8	33.7 ± 2.8	30.0 ± 1.4
HeP2	18.8 ± 4.2	17.9 ± 2.3	22.9 ± 1.2	8.6 ± 2.5
L132	7.9 ± 2.3	14.9 ± 2.5	25.9 ± 1.7	19.4 ± 2.9
PA-1	6.0 ± 1.4	22.6 ± 3.6	37.8 ± 2.2	26.0 ± 3.9
ECV304	23.9 ± 2.2	24.9 ± 2.4	27.9 ± 3.2	16.9 ± 1.3

DT-M1

	Percentage cytotoxicity at different concentrations			
Cell Line	1μM	100n M	10 nM	1nM
PTC	30 ± 3.9	31 ± 4.6	25 ± 3.6	26 ± 0.5
KB	12.9 ± 1.6	19.0 ± 3.2	23.9 ± 3.1	21.0 ± 26
U87MG	5.0 ± 2.5	32.2 ± 2.2	44.9 ± 2.7	29.4 ± 1.2
HBL100	29.6 ± 2.3	30.4 ± 2.4	31.6 ± 3.8	21.9 ± 2.4
HeP2	12.9 ± 1.2	22.9 ± 1.3	18.7 ± 2.2	15.8 ± 2.2
L132	9.6 ± 2.4	16.8 ± 2.1	26.7 ± 1.1	10.6 ± 1.9
PA-1	15.7 ± 1.2	25.9 ± 3.3	42.0 ± 2.2	27.5 ± 2.9
ECV304	17.7 ± 1.2	22.9 ± 1.0	16.9 ± 3.1	21.9 ± 1.6

DT-P1

	Percentage cytotoxicity at different concentrations			
Cell Line	1μM	100n M	10 nM	1nM
PTC	31±3.1	33±1.1	21±1.2	27±3.1
KB	18.0 ± 2.6	23.0 ± 2.2	32.0 ± 2.1	21.9 ± 2.6
U87MG	18.4 ± 2.4	32.9 ± 2.5	34.0 ± 2.6	9.6 ± 1.8
HBL100	28.9 ± 1.3	33.3 ± 1.4	34.9 ± 3.6	25.7 ± 2.5
HeP2	14.9 ± 1.3	28.9 ± 1.7	24.9 ± 2.8	13.9 ± 1.2
L132	17.8 ± 2.2	19.6 ± 1.1	29.0 ± 1.2	10.9 ± 1.9
PA-1	21.7 ± 2.2	25.6 ± 3.3	21.9 ± 2.2	20.5 ± 1.9
ECV304	25.9 ± 1.4	31.9 ± 1.3	19.9 ± 3.4	18.8 ± 2.6

EXAMPLE 7

In vivo activity of Lipo-peptide analogs

The antitumor activity of DT-B1 (SEQ ID NO: 3) was studied in human colon adenocarcinoma (PTC) xenografts in nude mice. PTC tumor xenografts were grown in Balb/c a thymic mice by subcutaneous inoculation of a single cell suspension of PTC cells (15 X 10^6 cells/100 μL). The tumor bearing animals were divided into 2 groups of three animals each including one group

comprising untreated control animals. Treatment with DT-B1 was initiated when the average tumor volumes, as measured using a vernier caliper, were between 1.3 cm^3. Solutions of DT-B1 was prepared at a concentration of 126 μg/ml and intravenously administered to the assigned group of tumor bearing animals at a dose of 12.6 μg/100 μL twice a day so that the total dose of 25.2μg/day was administered to each animal. The treatment was continued for a period of 14 days.

The antitumor activity of the compounds was monitored by measuring tumor volumes every fourth day using the formula W*W*L*0.4 (W = smaller diameter, L = larger diameter). The percentage inhibition of tumor growth was calculated using the formula (1- tumor volume-treated/tumor volume-control) * 100. Figure 1 shows the tumor kinetics till day 20 in the treated and untreated animals. DT-B1 showed a significant antitumor activity on PTC xenografts. The percentage inhibition of tumor growth caused by DT-B1 as compared to controls on day 20 was 95.85%.

All publications referenced are incorporated by reference herein, including the amino acid sequences listed in each publication. All the compounds disclosed and referred to in the publications mentioned above are incorporated by reference herein, including those compounds disclosed and referred to in articles cited by the 10 publications mentioned.

CLAIMS

1. A peptide derivative of the formula

X-Leu-Met-Tyr-Pro-Thr-Tyr-Leu-Lys-Y

wherein, X is acetyl or straight, branched, or cyclic alkanoyl group from 3-16 carbon atoms and

Y is a carboxy terminal residue selected from OH or amino; or a pharmaceutical acceptable salt of the peptide.

2. A peptide derivative of claim 1, wherein the alkanoyl groups is selected from acetyl, n-butanoyl, n-hexanoyl, n-octanoyl, lauroyl, myristoyl, palmitoyl, isohexanoyl, cyclohexanoyl, cyclopentylcarbonyl, n-heptanoyl, n-decanoyl, n-undecanoyl, or 3,7-dimethyloctanoyl.

3. A peptide derivative of claim 1, wherein X is Acetyl and the peptide is:

Acetyl-Leu-Met-Tyr-Pro-Thr-Tyr-Leu-Lys-OH (SEQ ID NO: 2)

or a pharmaceutically acceptable salt thereof.

4. A peptide derivative of claim 1, wherein X is butanoyl and the peptide is:

n-Butanoyl-Leu-Met-Tyr-Pro-Thr-Tyr-Leu-Lys-OH (SEQ ID NO: 3)

or a pharmaceutically acceptable salt thereof.

5. A peptide derivative of claim 1, wherein X is n-octanoyl and the peptide is:

n-Octanoyl-Leu-Met-Tyr-Pro-Thr-Tyr-Leu-Lys-OH (SEQ ID NO: 4)

or a pharmaceutically acceptable salt thereof

6. A peptide derivative of claim 1, wherein X is Myristoyl and the peptide is:

Myristoyl-Leu-Met-Tyr-Pro-Thr-Tyr-Leu-Lys-OH (SEQ ID NO: 5)

or a pharmaceutically acceptable salt thereof.

7. A peptide derivative of claim 1, wherein X is Palmitoyl and the peptide is:

Palmitoyl-Leu-Met-Tyr-Pro-Thr-Tyr-Leu-Lys-OH (SEQ ID NO: 6)]

or a pharmaceutically acceptable salt thereof.

8. A composition comprising an effective amount of a polypeptide

according to claim 1, and a pharmaceutically acceptable carrier.

9. A method of treatment of cancer in mammals which comprises the administration of an effective amount of polypeptide according to claim 1, alone or in combination with other polypeptides or anticancer compounds.

10. A solid phase synthesis process for preparation of a peptide analog of the formula:

X-Leu-Met-Tyr-Pro-Thr-Tyr-Leu-Lys-Y

wherein X is acetyl or straight, branched, or cyclic alkanoyl group from 3 - 16 carbon atoms and Y is a carboxy terminal residue selected from OH or amino; or a pharmaceutical acceptable salt of the peptide which comprises sequentially loading protected amino acids in sequential cycles to the amino terminus of a solid phase resin, coupling the amino acids to assemble a peptide-resin assembly, removing the protecting groups and cleaving the peptide from the resin to obtain a peptide.

11. The process as claimed in claim 10, wherein the coupling is carried out in the presence of activating agents selected from the group consisting of DCC, DIPCDI, DIEA, BOP, PyBOP, HBTU, TBTU, and HOBt.

12. The process as claimed in claim 10, wherein the coupling was carried out in the presence of a solvent selected from the group consisting of DMF, DCM, NMP or any mixtures thereof.

13. A process as claimed in claim 10, wherein said crude peptide is cleaved from said peptide-resin assembly by treatment with trifluoroacetic acid, crystalline phenol, ethanedithiol, thioanisole and water for 1.5 to 5 hours at room temperature.

ABSTRACT

This invention relates to novel antiproliferative and anti secrectory peptides that are inhibitory to vasoactive intestinal peptide receptor and are useful in the treatment of cancer. The invention particularly relates to the synthesis of lipid-peptide conjugates containing fatty acids of different sizes, which inhibits the binding of VIP to its receptors. The invention encompasses methods for generation of these peptides, composition containing these peptides and the pharmacological applications of these peptides especially in the treatment and prevention of cancer.

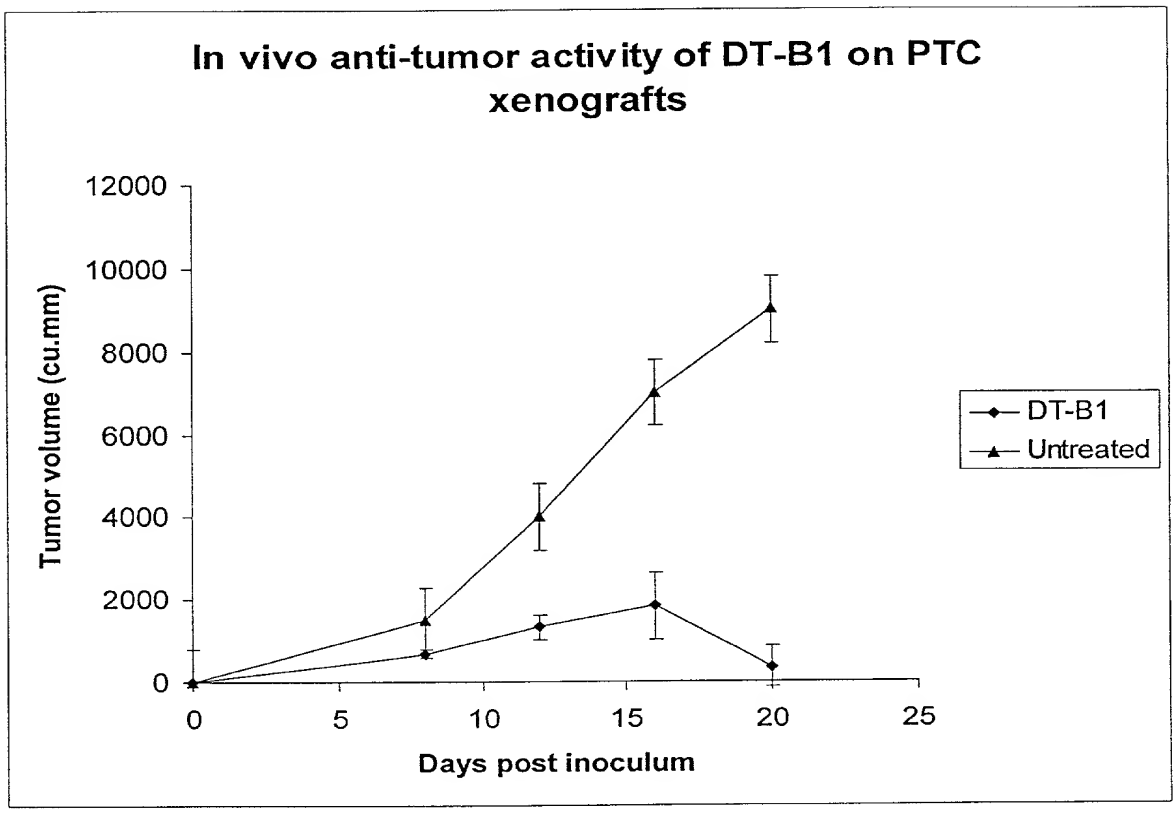

In vivo anti-tumor activity of DT-B1 on PTC xenografts
Tumor volume (cu.mm)
12000
10000
8000
6000
4000
2000
0
0
5
10
15
20
25
Days post inoculum
DT-B1
Untreated

Figure. 1

Practitioner's Docket No. U 012858-1 ***PATENT***

COMBINED DECLARATION AND POWER OF ATTORNEY

(ORIGINAL, DESIGN, NATIONAL STAGE OF PCT, SUPPLEMENTAL, DIVISIONAL, CONTINUATION, OR C-I-P)

As a below named inventor, I hereby declare that:

TYPE OF DECLARATION

This declaration is of the following type:

(check one applicable item below)

[X] original.
[ ] design.

NOTE: With the exception of a supplemental oath or declaration submitted in a reissue, a supplemental oath or declaration is not treated as an amendment under 37 CFR 1.312 (Amendments after allowance). M.P.E.P. Section 714.16, 7th Ed.

[ ] supplemental.

NOTE: If the declaration is for an International Application being filed as a divisional, continuation or continuation-in-part application, do not check next item; check appropriate one of last three items.

[ ] national stage of PCT.

NOTE: If one of the following 3 items apply, then complete and also attach ADDED PAGES FOR DIVISIONAL, CONTINUATION OR C-I-P.

NOTE: See 37 C.F.R. Section 1.63(d) (continued prosecution application) for use of a prior nonprovisional application declaration in the continuation or divisional application being filed on behalf of the same or fewer of the inventors named in the prior application.

[ ] divisional.
[ ] continuation.

NOTE: Where an application discloses and claims subject matter not disclosed in the prior application, or a continuation or divisional application names an inventor not named in the prior application, a continuation-in-part application must be filed under 37 C.F.R. Section 1.53(b) (application filing requirements-nonprovisional application).

[ ] continuation-in-part (C-I-P).

INVENTORSHIP IDENTIFICATION

WARNING: If the inventors are each not the inventors of all the claims, an explanation of the facts, including the ownership of all the claims at the time the last claimed invention was made, should be submitted.

My residence, post office address and citizenship are as stated below, next to my name. I believe that I am the original, first and sole inventor *(if only one name is listed below)* or an original, first and joint inventor *(if plural names are listed below)* of the subject matter that is claimed, and for which a patent is sought on the invention entitled:

TITLE OF INVENTION

NOVEL PEPTIDES FOR TREATMENT OF CANCER

SPECIFICATION IDENTIFICATION

The specification of which:

(complete (a), (b), or (c))

(a) [X] is attached hereto.

NOTE: "The following combinations of information supplied in an oath or declaration filed on the application filing date with a specification are acceptable as minimums for identifying a specification and compliance with any one of the items below will be accepted as complying with the identification requirement of 37 C.F.R. Section 1.63:

"(1) name of inventor(s), and reference to an attached specification which is both attached to the oath or declaration at the time of execution and submitted with the oath or declaration on filing;

"(2) name of inventor(s), and attorney docket number which was on the specification as filed; or

"(3) name of inventor(s), and title which was on the specification as filed."

Notice of July 13, 1995 (1177 O.G. 60).

(b) [ ] was filed on _____, [ ] as Application No. _____
[ ] and was amended on _____ (if applicable).

NOTE: Amendments filed after the original papers are deposited with the PTO that contain new matter are not accorded a filing date by being referred to in the declaration. Accordingly, the amendments involved are those filed with the application papers or, in the case of a supplemental declaration, are those amendments claiming matter not encompassed in the original statement of invention or claims. See 37 C.F.R. Section 1.67.

NOTE: "The following combinations of information supplied in an oath or declaration filed after the filing date are acceptable as minimums for identifying a specification and compliance with any one of the items below will be accepted as complying with the identification requirement of 37 C.F.R. Section 1.63:

(A) application number (consisting of the series code and the serial number, e.g., 08/123,456);
(B) serial number and filing date;
(C) attorney docket number which was on the specification as filed;
(D) title which was on the specification as filed and reference to an attached specification which is both attached to the oath or declaration at the time of execution and submitted with the oath or declaration; or
(E) title which was on the specification as filed and accompanied by a cover letter accurately identifying the application for which it was intended by either the application number (consisting of the series code and the serial number, e.g., 08/123,456), or serial number and filing date. Absent any statement(s) to the contrary, it will be presumed that the application filed in the PTO is the application which the inventor(s) executed by signing the oath or declaration.

M.P.E.P. Section 601.01(a), 7th ed.

(c) [ ] was described and claimed in PCT International Application No. ____________ filed on __________ and as amended under PCT Article 19 on ___________*(if any).*

SUPPLEMENTAL DECLARATION (37 C.F.R. Section 1.67(b))

(complete the following where a supplemental declaration is being submitted)

[ ] I hereby declare that the subject matter of the

[ ] attached amendment
[ ] amendment filed on _______________.

was part of my/our invention and was invented before the filing date of the original application, above identified, for such invention.

ACKNOWLEDGMENT OF REVIEW OF PAPERS AND DUTY OF CANDOR

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information, which is material to patentability as defined in 37, Code of Federal Regulations, Section 1.56,

(also check the following items, if desired)

[ ] and which is material to the examination of this application, namely, information where there is a substantial likelihood that a reasonable Examiner would consider it important in deciding whether to allow the application to issue as a patent, and

[ ] in compliance with this duty, there is attached an information disclosure statement, in accordance with 37 C.F.R. Section 1.98.

PRIORITY CLAIM (35 U.S.C. Section 119(a)-(d))

NOTE: "The claim to priority need be in no special form and may be made by the attorney or agent if the foreign application is referred to in the oath or declaration as required by Section 1.63. The claim for priority and the certified copy of the foreign application specified in 35 U.S.C. Section 119(b) must be filed in the case of an interference (Section 1.630), when necessary to overcome the date of a reference relied upon by the examiner, when specifically required by the examiner, and in all other situations, before the patent is granted. If the claim for priority or the certified copy of the foreign application is filed after the date the issue fee is paid, it must be accompanied by a petition requesting entry and by the fee set forth in Section 1.17(i). If the certified copy is not in the English language, a translation need not be filed except in the case of interference; or when necessary to overcome the date of a reference relied upon by the examiner; or when specifically required by the examiner, in which event an English language translation must be filed together with a statement that the translation of the certified copy is accurate." 37 C.F.R. Section 1.55(a).

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119(a)-(d) of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed.

(complete (d) or (e))

(d) [ ] no such applications have been filed.
(e) [ ] such applications have been filed as follows.

NOTE: Where item (c) is entered above and the International Application which designated the U.S. itself claimed priority check item (e), enter the details below and make the priority claim.

PRIOR FOREIGN/PCT APPLICATION(S) FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS APPLICATION AND ANY PRIORITY CLAIMS UNDER 35 U.S.C. SECTION 119(a)-(d)

COUNTRY (OR INDICATE IF PCT)	APPLICATION NUMBER	DATE OF FILING DAY, MONTH, YEAR	PRIORITY CLAIMED UNDER 35 USC 119
			[ ]YES [ ]NO
			[ ]YES [ ]NO
			[ ]YES [ ]NO
			[ ]YES [ ]NO
			[ ]YES [ ]NO

CLAIM FOR BENEFIT OF PRIOR U.S. PROVISIONAL APPLICATION(S)
(35 U.S.C. Section 119(e))

I hereby claim the benefit under Title 35, United States Code, Section 119(e) of any United States provisional application(s) listed below:

PROVISIONAL APPLICATION NUMBER	FILING DATE
______ / ______________________	__________
______ / ______________________	__________
______ / ______________________	__________

CLAIM FOR BENEFIT OF EARLIER U.S./PCT APPLICATION(S) UNDER 35 U.S.C. SECTION 120

[ ] The claim for the benefit of any such applications are set forth in the attached ADDED PAGES TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR DIVISIONAL, CONTINUATION OR CONTINUATION-IN-PART (C-I-P) APPLICATION.

ALL FOREIGN APPLICATION(S), *IF ANY*, FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

____

____

____

NOTE: *If the application filed more than 12 months from the filing date of this application is a PCT filing forming the basis for this application entering the United States as (1) the national stage, or (2) a continuation, divisional, or continuation-in-part, then also complete ADDED PAGES TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR DIVISIONAL, CONTINUATION OR C-I-P APPLICATION for benefit of the prior U.S. or PCT application(s) under 35 U.S.C. Section 120.*

POWER OF ATTORNEY

I hereby appoint the following practitioner(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

(list name and registration number)

JOSEPH H. HANDELMAN, 26179
JOHN RICHARDS, 31053
RICHARD J. STREIT, 25765
PETER D. GALLOWAY, 27885
IAN C. BAILLIE, 24090
THOMAS F. PETERSON, 24790

RICHARD P. BERG, 28145
JULIAN H. COHEN, 20302
WILLIAM R. EVANS 25858
JANET I. CORD, 33778
CLIFFORD J. MASS, 30086
CYNTHIA R. MILLER, 34678

(Check the following item, if applicable)

[ ] I hereby appoint the practitioner(s) associated with the Customer Number provided below to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

[ ] Attached, as part of this declaration and power of attorney, is the authorization of the above-named practitioner(s) to accept and follow instructions from my representative(s).

NOTE: "Special care should be taken in continuation or divisional applications to ensure that any change of correspondence address in a prior application is reflected in the continuation or divisional application. For example, where a copy of the oath or declaration from the prior application is submitted for a continuation or divisional application filed under 37 CFR 1.53(b) and the copy of the oath or declaration from the prior application designates an old correspondence address, the Office may not recognize, in the continuation or divisional application, the change of correspondence address made during the prosecution of the prior application. Applicant is required to identify the change of correspondence address in the continuation or divisional application to ensure that communications from the Office are mailed to the current correspondence address. 37 CFR 1.63(d)(4)." Section 601.03, M.P.E.P., 7th Ed.

SEND CORRESPONDENCE TO

Ladas & Parry
26 West 61st Street
New York, N.Y. 10023

DIRECT TELEPHONE CALLS TO:
(Name and telephone number)

(212)708-1935

(complete the following if applicable)

Since this filing is a [ ] continuation [ ] divisional there is attached hereto a Change of Correspondence Address so that there will be no question as to where the PTO should direct all correspondence.

DECLARATION

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

SIGNATURE(S)

NOTE: Carefully indicate the family (or last) name, as it should appear on the filing receipt and all other document.

NOTE: Each inventor must be identified by full name, including the family name, and at least one given name without abbreviation together with any other given name or initial, and by his/her residence, post office address and country of citizenship. 37 C.F.R. Section 1.63(a)(3).

NOTE: Inventors may execute separate declarations/oaths provided each declaration/oath sets forth all the inventors. Section 1.63(a)(3) requires that a declaration/oath, inter alia, identify each inventor and prohibits the execution of separate declarations/oaths which each sets forth only the name of the executing inventor. 62 Fed. Reg. 53,131, 53,142, October 10, 1997.

Full name of sole or first inventor

ANAND — C. — BURMAN
(Given Name) *(Middle Initial or Name)* *Family (Or Last Name)*

Inventor's signature (✓) Anand C. Burman

Date (✓) 22.7.2000 **Country of Citizenship** INDIA

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Post Office Address C/O DABUR RESEARCH FOUNDATION
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Full name of second joint inventor, if any

SUDHANAND — — PRASAD
(Given Name) *(Middle Initial or Name)* *Family (Or Last Name)*

Inventor's signature (✓) Sudhanand Prasad

Date (✓) 22/7/2000 **Country of Citizenship** INDIA

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Full name of third joint inventor, if any

RAMA — — MUKHERJEE
(Given Name) *(Middle Initial or Name)* *Family (Or Last Name)*

Inventor's signature(✓) R Mukherjee

Date (✓) 22/7/2000 **Country of Citizenship** INDIA

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(check proper box(es) for any of the following added page(s) that form a part of this declaration)

[X] **Signature** for fourth and subsequent joint inventors. *Number of pages added* ____1_____

* * *

[ ] **Signature** by administrator(trix), executor(trix) or legal representative for deceased or incapacitated inventor. *Number of pages added* _____

* * *

[ ] **Signature** for inventor who refuses to sign or cannot be reached by person authorized under 37 C.F.R. Section 1.47. *Number of pages added* _____

* * *

[ ] Added page for **signature** by one joint inventor on behalf of deceased inventor(s) where legal representative cannot be appointed in time. (37 C.F.R. Section 1.47)

* * *

[ ] Added pages to combined declaration and power of attorney for divisional, continuation, or continuation-in-part (C-I-P) application.

[ ] Number of pages added _____

* * *

[ ] Authorization of practitioner(s) to accept and follow instructions from representative.

(If no further pages form a part of this Declaration, then end this Declaration with this page and check the following item)

[ ] This declaration ends with this page.

Practitioner's Docket No. U 012858-1

ADDED PAGE TO COMBINED DECLARATION AND POWER OF ATTORNEY FOR SIGNATURE BY FOURTH AND SUBSEQUENT INVENTORS

Full name of fourth joint inventor, if any

MANU (Given Name) _____ (Middle Initial or Name) JAGGI Family (Or Last Name)

Inventor's signature(✓) Manu Jaggi

Date (✓) 22.7.2000 **Country of Citizenship** INDIA

Residence GHAZIABAD, INDIA

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Full name of fifth joint inventor, if any

ANU (Given Name) T. (Middle Initial or Name) SINGH Family (Or Last Name)

Inventor's signature(✓) A T Singh

Date (✓) 22-7-2000 **Country of Citizenship** INDIA

Residence GHAZIABAD, INDIA

Post Office Address C/O DABUR RESEARCH FOUNDATION

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Full name of sixth joint inventor, if any

RAJAN (Given Name) _____ (Middle Initial or Name) SHARMA Family (Or Last Name)

Inventor's signature(✓) Rajan Sharma

Date (✓) 22.7.2000 **Country of Citizenship** INDIA

Residence GHAZIABAD, INDIA

Post Office Address C/O DABUR RESEARCH FOUNDATION

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SEQUENCE LISTING

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PRASAD, SUDHANAND
MUKHERJEE, RAMA
JAGGI, MANU
SINGH T, ANU
SHARMA, RAJAN

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